SYNTHESIS AND CHARACTERISATION OF SILVER NANOPARTICLES USING SEED EXTRACT OF *ZANTHOXYLUM ARMATUM* FOR REMEDIATION OF DYE & HEAVY METAL CONTAMINATED TEXTILE EFFLUENTS

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CERTIFICATE

This is certified that synopsis of Anju Bhardwaj, Ph.D. Chemistry student of School of Sciences (SoS) has been finalised in consultation with research guiding committee. The student has successfully delivered his/her synopsis seminar entitled "Synthesis and Characterisation of Silver Nanoparticles using Seed Extract of *Zanthoxylum armatum* for Remediation of Dye & Heavy Metal Contaminated Textile Effluents" on 24 August 2023 in accordance with the academic regulations. The suggestions given by the members have been duly incorporated.

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1. INTRODUCTION

The secret to sustaining life on earth is fresh water. Aquatic resources are becoming increasingly valuable as a result of human civilization, population growth, industrial revolution and fast urbanization. When dangerous substances—often chemicals or microorganisms—contaminate a stream, river, lake, ocean, aquifer, or other body of water, its quality deteriorates and they become poisonous to both people and the environment. This is known as water pollution. Our health is in danger due to the prevalent issue of water pollution. More people are killed each year by unsafe water than by war and all other types of violence combined. Due to limited sources of drinkable water, we can actually access only 1% of the freshwater on the planet. By 2050, when the world's demand for freshwater is anticipated to be one-third higher than it is now, the problems would aggravate, if nothing is done now. There are various factors that deteriorate the quality of water. Some of them are listed below.

1.1 Causes of water contamination

1.1.1 Agricultural

Using over 70% of the world's surface water for farming and livestock production, the agricultural sector is not only the biggest consumer of freshwater resources but also a significant polluter of the environment. Every time it rains, nutrients and pathogens from farms, livestock operations & insecticides, fertilizers, and animal waste are washed into our waterways. Algal blooms, a toxic soup of blue-green algae are one of the most serious threats to water quality in the world produced by too much nitrogen and phosphorus in water or the air.

1.1.2 Washed-up garbage and sewage

Wastewater is reclaimed water that originates from toilets, sinks and showers as well as from commercial, industrial and agricultural activity. The phrase also refers to storm water runoff formed when rainwater flushes chemicals, oil, grease, and other substances from impermeable surfaces into our waterways.

1.1.3 Oil spills

The estimated 1 million tons of oil that enter marine habitats annually come from sources on land, such as industry, farms and cities rather than from tanker disasters.

1.1.4 Industrial waste

The environment may suffer long-term effects from industrial water pollution. In a variety of industrial operations, water is used and is in touch with poisonous materials, heavy metals, organic sludge, and even radioactive sludge. Therefore, when such contaminated water is dumped into the ocean or other bodies of water without being treated, they become unfit for any use by humans or agriculture.

1.1.5 Radioactive elements

Any pollutant that emits radiation over and above what the environment naturally releases is considered radioactive waste. It is produced by uranium mining, nuclear power plants, during the development and testing of military weapons, and educational institutions that use radioactive materials for study and medical treatment. Since radioactive waste can stay in the environment for tens of thousands of years, its disposal is incredibly challenging.

There is no denying that nature has an amazing ability to deal with tiny amounts of wastewater and water pollution, but it would be risky if a large amount of untreated effluent were to be released into natural water bodies. More than 8000 synthetic chemical compounds are reportedly connected to various dye production stages (Bhatia *et al.,* 2017).

1.2 Textile industries

In 2018–19, the Indian textile and clothing sector controlled 5% of global textile and apparel commerce, contributed 2% to GDP, and earned 12% from exports. The value of textile exports between April 2021 and October 2021 was US$ 22.89 billion.

Punjab has been among the top producers of cotton in India since 1970 (Hitctuings *et al.*, 1980). Ludhiana is one stop destination for woollen and cotton clothes. The spectacular rise in the textile industry of Ludhiana can be attributed to its geographic location and proximity

to other important trading cities. Similar to how Delhi is regarded as the centre for ready-made clothing, Ludhiana is regarded as the centre for hosiery and textile machinery.

The city of Ludhiana is made up of sizable industrial sectors, with numerous key locations housing industrial facilities. The city's core is home to many textile processing facilities, with the main hubs being Sherpur Road, Mothi Nagar, Rahon Road, Sunder Nagar, and Jalandhar Bye Pass.

The textile industry uses a significant amount of water and chemicals, and it also supplies significant amounts of colorants and other chemicals. Even the lowest concentration of the dye i.e. 1 ppm is visible through water. The improper dye uptake as well as the degree of fixation on the substrate, which is governed by several factors such as application method, material-to-liquor ratio, pH etc are one of the main causes for the release of both water-insoluble and water-soluble dyes in the wastewaters.

The increase in industrialization has raised concerns about environmental issues (Suri *et al.,* 2023). The augmentation of biochemical oxygen demand (BOD), chemical oxygen demand (COD), pH and suspended particles in water bodies is the fundamental issue of effluent from the textile industry with environmental difficulties (Wang *et al.*, 2011).

Textile effluent must be treated before being discharged into waterways because it contains pollutants that have a significant negative impact on the environment such as solids, oils, metal complexes, high levels of organics, complex synthetic dyes and leftovers from pre-processing steps used to prepare and colour fabrics. Because it offers a significant risk to the environment and human health, the pollution brought on by textile effluents has thus become a severe issue (Sarker *et al.,* 2015)

The major aesthetic problem of the textile effluent is colour, due to the voluminous use of synthetic organic compounds as dyes. Chromophores, having an azo bond (–N=N–), may be mono-azo, di-azo, and poly-azo; or –C=O; –N=O, etc., are responsible for colouring properties of dyes.The remittance of untreated textile effluent including liquid, gaseous, and solid wastes leads to severe environmental problems, affecting the aquatic ecosystem by reducing the penetration of sunlight and thus decreasing the rate of photosynthesis and increasing biological oxygen demand (Puvaneshwari *et al.,* 2014).The natural stream of

water, in turn, leads to a fatal effect on fish and suppresses the growth of microorganisms (Khan *et al.,* 2014).

The waste products released by the textile industries are of environmental concern since they reduce the existence of hydrogen sulphide group chemicals, which lower oxygen concentration in water. In addition, it prevents light from entering the water body and is detrimental to the ecosystem of water (Rawat *et al.,* 2016).

Human diseases are brought on by textile waste. Chlorine, a recognized carcinogen, is linked to organic molecules in about 40% of textile dyes. Before a kid is born, allergic reactions to chemicals can be caused by evaporating chemicals into the air and absorbing them through our skin. Cells' regular operation is interfered with as a result of this chemical pollution. As a result, critical processes including respiration, osmoregulation, reproduction, and even mortality may be hampered, which could change the physiology and biochemical systems of animals (D Dadi *et al.,* 2017).

As a chromophore group found in textile dyes, heavy metals cannot be broken down by the body, thus they build up in the body's key organs and produce a variety of disease symptoms (Chung *et al.,* 2016).Therefore, due to the detrimental effects on the natural ecosystem and long-term health effects, untreated or improperly treated textile effluent may harm both aquatic and terrestrial species (Rajpal *et al.,* 2013).

1.3 What is research about?

The present study is focussed on the analysis of various physico- chemical and biological parameters of effluents from textile industries situated in Ludhiana and the effluent which is drained into the Buddha Nullah.

Buddha Nullah is a seasonal water stream that runs the entire length of Punjab's Malwa district. It drains once it has travelled through the densely populated city of Ludhiana into the Indus River's tributary, the Sutlej River. It has since become a significant source of pollution in the area, in addition to the main Sutlej River because it gets contaminated after entering the industrialized and densely populated city of Ludhiana. Chemicals, different dyes, cyanide, chromium, nickel and other heavy metal traces find their way into the Buddha Nullah.

Buddha Nullah of Ludhiana is a prime source of pollution due to the discharge of industrial effluents, domestic and agricultural waste and other anthropogenic activities around the Nullah which might be harmful to the groundwater as well as to humans and animals (de Almeida *et al.,* 2019). Numerous textile mills next to Buddha Nullah discharge waste into the body of water, which contribute significantly to water pollution. Since the characteristics of wastewater are ever-changing, this study has been undertaken to assess the effluent attributes of the textile industries of Ludhiana, which are in the vicinity of Buddha Nullah.

The production of clothing requires 20,000 chemicals, many of which are carcinogenic and account for one-fifth of all industrial pollution in the globe. The discharge of colour from these textile companies contaminates textile wastewater, which results in highly polluted effluent in significant proportions, raising concerns about water pollution.

Release of dyes directly into water bodies without treatment stopped the penetration of sunlight into the deeper layers of the water, altering photosynthetic activity, deteriorating water quality, reducing the solubility of oxygen, and exerting toxic effects on aquatic flora and fauna (Bhatia *et al.,* 2018).The textile industries use more water in comparison of other industries and are likely to produce highly polluted wastewater (Carneiro *et al.,* 2010).

The textile business also contributes to air pollution since it uses diesel generators, boilers and thermal packs. The pollutants such as Suspended Particulate Matter (SPM), sulphur dioxide (SO_2) gas, oxide of nitrogen gas etc are generated.

Additionally, industrial effluent has significant nitrogen content, including organic nitrogen, nitrate and nitrite. Significant water pollution is considered to be caused by the presence of nitrogen in wastewater. When nitrogen is present in drinking water, it poses a major risk to one's health.

Numerous types of dyes are used in the textile industry. Major synthetic dyes are the azo type which includes reactive, dispersed and acid dyes. The majority of these synthetic dyes are water-soluble but some dyes show hydrophobic behaviour such as dispersed dyes used for polyester fabrics.

Disperse dyes are non-ionic aromatic compounds bearing azo or anthraquinone as a chromophore group. The dyes are applied to the fibers by stable aqueous dispersion containing auxiliaries, especially dispersants at high temperatures (Mor *et al.,* 2018). Methylene blue is the most commonly used basic azo dye in the textile industry which exhibits toxic effects on inhalation and shortness of breath, and if ingested may cause burning sensation, nausea, vomiting, dizziness, headache, discoloration of urine, and methemoglobinemia.(Singh *et al.,* 2013)

1.4 What is known ?

India's Industrial development coupled with urban development is generating huge quantities of untreated wastewater leading to increased water pollution and human diseases. Punjab - An agriculturally advanced state of the Indian Union, where land man ratio is declining at a rapid pace due to population explosion and urbanization is also facing a steady decline and deterioration in its natural resources.

Numerous investigations on how urbanization has affected the groundwater quality and course of natural drainage have been carried out (Khan *et al.,* 2019) However the assessment of wastewater through various chemical methods requires the standardization of thousands of organic pollutants present in the environment, making them tedious and time-consuming processes (Kaur *et al.,* 2017 and Brraich *et al.*, 2021).

It was found that pollution caused by heavy metals is not only making the water of Buddha nullah unfit for human intake but also noxious for aquatic organisms and agricultural practices (Bhuiyan *et al.*, 2016).

Aquatic life and vegetation growth are being hampered by pollution from textile effluents. As soon as these effluents are released into an aquatic environment, the heavy metals enter the food chain, where they cause a variety of health issues in people. Despite the fact that the Central Pollution Control Board (CPCB) has severe regulations that apply to all Indian enterprises, the environment is still not in a good state.

2. REVIEW OF LITERATURE

2.1 Textile sector as a national economic pillar

The oldest industries in India are those related to textiles. The demand for textiles has increased during the previous five years. The sector (including printing and dye) attracted US$3.44 billion in foreign direct investment (FDI) between April 2000 and March 2020. The Indian textile sector has a promising future, helped by strong domestic demand and export demand (IBEF, 2020).

2.2 Processes in the textile industry

Water is heavily utilized in the textile industries (Shaker *et al.,* 2016, Patel et al., 2015). Pre-processing (pre-treatment), processing (dyeing and printing), and post-processing (finishing) are the various processes of the textile wet-processing (TWP) business. In the wet processing of textiles, chemicals such as formaldehyde, acids, thickeners, and pigments are utilized. The chemicals mentioned above have a negative impact on human health and seriously contaminate water supplies (Wang *et al.,* 2022).

In textile wastewater, the majority of the organic stuff is not biodegradable. As a result, it is challenging to break down these chemicals using biotreatment techniques. It has been suggested that several treatment method combinations can be employed to treat wastewater generated by the textile sector. Wastewater from the textile industry has been treated using CF-NF, AOPs, and MFC combinations. In the textile industry, selecting the most efficient and affordable treatment processes, or their combinations, based on the dyestuffs and dyeing techniques used during production is generally practiced (Raval *et al.,* 2016).

In the 21st century, numerous scientific subjects have advanced significantly. Over the past 20 years, extensive research has been conducted on a wide range of problems in the domains of environmental science and engineering. The paper, rubber, leather, printing, textile, cosmetics, and paper sectors all produce significant amounts of wastewater every day (Bhateria *et al.,* 2019).

The biochemical and physiological functioning of the body depends on the presence of heavy metals such as nickel (Ni), selenium (Si), cobalt (Co), iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg), manganese (Mn), and molybdenum (Mo). However, if these

metals are found in excess of their permissible levels, they have a disastrous impact on both people and the environment. Heavy metals are non-biodegradable and therefore have a long persistence in living systems (Diagomanolin *et al.,* 2004) As a result, the United States Environmental Protection Agency (USEPA) has established regulatory standards for the discharge of heavy metals in wastewater and drinking water (O' Grady *et al.,* 2011 and Younis *et al.,* 2021). These harmful metal ions have the potential to harm life systems in a certain way that cannot be repaired.

The large amount of water needed to produce textiles is itself a major concern for the scientific community. The lithosphere, hydrosphere and atmosphere are key abiotic components of the ecosystem and are all physically affected by the discharge of wastewater from the textile factories that contain harmful chemicals (Christie *et al.,* 2007).

Total dissolved solids in the generated effluent increase as a result of the massive amount of salt utilized during the dyeing process. Synthetic dyes can contain a wide range of various active compounds and heavy metals (Ajmal *et al.,* 2014) The numerous auxiliaries used in dyeing do not disintegrate, making it impossible to recycle them and increasing biological oxygen demand (BOD). As a result, prolonged exposure to these substances may have negative long-term effects on both human health and the ecosystem. According to a study, the textile waste effluents release roughly 15% (or 1000 tons) of non-biodegradable and hazardous dyes into water bodies every year (Sajab *et al.,* 2011).

2.3 Impact on aquatic organisms and plants

When dye-containing effluent is dumped into water bodies, it increases the levels of dissolved and suspended particles as well as COD. As a result, it prevents light from entering the water, which has an impact on microbial growth, aquatic toxicity, and photosynthesis in aquatic plants (Strickland *et al.,* 1995).

Dyes containing aromatic chemical groups make water bodies more resistant to light and impair environmental conditions in general. Infrared/ultraviolet light is now absorbed by the linked chemical (Anjaneyulu *et al.,* 2005 and Pirkarami *et al.,* 2013).

Because the hazardous textile effluent contains various heavy metals and chlorine, it poisons aquatic life and consumes oxygen from the water (Mukhopadhyay *et al.,* 2011).

This has an impact on the ability of microscopic organisms like algae to produce food and oxygen. By clogging the gills, textile effluent with suspended particles can interfere with fish breathing. Azo dyes also interfere with procedures used to remediate wastewater, such as ultraviolet decontamination (Midha *et al.,* 2008).

Bad odours are produced by textile wastewater (Kaupaie *et al.,* 2013) High biological oxygen demand, which results in obstruction in physiochemical and biological attack, is caused by the formation of hazardous by-products like amines (Reemtsma *et al.*, 2006, Khan *et al.*, 2014) and (Karimifard *et al.*, 2016)

2.4 Impact on human health

Dyes are synthetic and have an aromatic structure, which makes them carcinogenic and non-biodegradable and contributes to allergic dermatitis and other skin conditions. Different kinds of azo group-containing pigments are poisonous both chronically and acutely. Amines, which are the breakdown products of azo dyes, pose a serious risk to the health of several organs, including the skin, lungs, and gastrointestinal (GI) tract, in addition to causing haemoglobin adducts and problems with blood formation (Vasiraja *et al.*, 2023).

Additionally, carcinogens and mutagens that are hazardous to the human body are included in chlorine-based colours. These carcinogens are exposed to the air by chemical evaporation, whereupon they are inhaled, leading to eventual physiological and biochemical abnormalities in the body.

Heavy metals in wastewater may only be removed or modified chemically, which also affects their availability to the environment. if not already deleted. Metals are either transported to the sludge and accumulate there or they go through the treatment system as effluent before entering a municipal treatment system.

Activated carbon derived from *Prosopis juliflora* stem was used to remove the methylene blue dye and heavy metal-containing textile industry effluent (Utami *et al.,* 2023). Photocatalytic removal of heavy metal and organic dye was done on N-doped TiO_2 on reduced graphene oxide (Bhangi *et al.,* 2022). Copper nanoparticles in a chitosan

entrapped copolymer matrix were used for the photocatalytic reduction of textile dye and adsorption of heavy metal from water (Joseph *et al.*, 2019).

Mercury, lead, chromium, and arsenic are just a few of the dangerous heavy metals to which people are exposed. The health effects, sources and the maximum permissible concentration of some of the common heavy metals is mentioned in Table 1.

Table 1 - Characteristics of Common Heavy Metals (Kaur *et al.*, 2017).

HEAVY METAL	HEALTH EFFECTS	SOURCES	MAXIMUM CONTAMINATION (USEPA)	MAXIMUM CONTAMINATION (WHO)
Arsenic (As)	Skin disease Issues with the circulatory system	Naturally occurring electronics production	0.010 mg L^{-1}	0.010 mg L^{-1}
Mercury (Hg)	Damage to kidney Damage to the nervous system	Fossil fuel combustion Electronic Industries	0.012 mg L^{-1}	0.006 mg L^{-1}
Cadmium (Cd)	Kidney damage Carcinogenic	Occur naturally chemical industries	0.005 mg L^{-1}	0.003 mg L^{-1}
Lead (Pb)	Reduced neural development Damages kidney	Lead-based products Household plumbing system.	0.0 mg L^{-1}	0.01 mg L^{-1}
Chromium (Cr)	Allergic dermatitis Diarrhoea, Nausea and vomiting.	Steel manufacturing industries Occur naturally	0.1 mg L^{-1}	0.05 mg L^{-1}
Copper (Cu)	Gastrointestinal issues,liver or kidney damage.	Household plumbing system Natural occurence	1.3 mg L^{-1}	2.0 mg L^{-1}

Values prescribed by United States Environmental Protection Agency (USEPA, 2019) and World Health Organisation (WHO, 2017)

2.5 Impact on the soil

Textile mill effluents are complex in nature because they contain a variety of dyes, metal contaminations, and other organic agents that can accumulate in natural sources under different operating conditions; soil and water after untreated disposal that may be toxic to ecosystems and human life; and the presence of xenobiotics elements in open and partially covered pits left behind by industrial or domestic activities alter chemical and physical properties of arable land.

As highly hazardous substances, Cr, As, and Cd have harmful effects on soil, crop output and the quality of agricultural goods. Plants begin to experience detrimental impacts when heavy metals are absorbed and stored in them.

The density of the slug and snail population at Buddha Nullah was found to be of sandy loam texture with high organic matter content, phosphorous, nitrogen, porosity and less bulk density (Kaur *et al.,* 2017).Higher concentrations of heavy metals were found in industrial soil followed by non-agricultural and agricultural soils (Tanveer *et al.,* 2022).

2.6 Treatment techniques for industrial wastewater

2.6.1 Physical treatment

Physical wastewater treatment procedures use physical processes to improve the quality of industrial wastewater. Sedimentation, aeration, degasification, filtering, screening, flotation with skimming, and equalization are a few of these techniques.

The physical treatment processes have an electrocoagulation system (ECS), lamella clarifier, dissolved air flotation (DAF) and reverse osmosis were employed (Spagnoli *et al.,* 2023).

Adsorption is a popular treatment option due to its inexpensive startup costs, straightforward operation, focused pollutant removal, and lack of reactive intermediate formation. As activated carbon for the adsorptive removal of dyes, low-cost waste materials like Jatropha seeds, cashew nut shells, and sugarcane bagasse have been chemically activated.(Haider *et al.,* 2011)

According to studies that have been published on the adsorptive removal of hydrolyzed dye compounds, specific functional groups in the dye structure are what cause the dye molecule to adhere to the adsorbent surface. The dye adsorption has been linked to a number of functional groups, including the sulphonic acid group and the NH_2 group (Azanav *et al.*, 2022)

2.6.2 Chemical treatment

These are the techniques for adding chemicals to wastewater that enhance water quality by causing chemical interactions between the additional chemical and the water contaminants. The treatment of industrial effluents has demonstrated significant potential for advanced oxidation processes.(Jahan *et al.*, 2022)

For the preliminary experiments in the current research, chemical activators such acetic acid, sulfuric acid, and phosphoric acid were used. The percentage of dye removed from synthetic textile effluent employing the adsorbents activated with acetic acid, sulphuric acid, and phosphoric acid, respectively, was found to be about 69%, 81% and 90%. The preliminary batch adsorption investigations yielded greater dye removal efficiencies, hence phosphoric acid was chosen for the chemical activation.

In comparison to more conventional approaches to dealing with wastewater, the cost of energy and maintenance is significantly higher. As a result, it is imperative that research and development efforts be focused on the creation of more practical economic procedures. Only a few ZLD techniques have been widely used.(Abbas *et al.*, 2022)

The textile effluents treated with immobilized ZnO NPS demonstrated a substantial reduction in pH, turbidity, COD, electrical conductivity (EC) and total suspended solids (TSS) in comparison to the untreated samples. Overall, the photocatalytic performance of biogenic ZnO NPs coated on Ca-Alg beads for the decolorization of MB and treatment of textile effluents is highlighted in this work (Tanveer *et al.*, 2022) Ozone, Fenton, and photo-Fenton processes are integrated with an electrocoagulation system for successful textile dye-bath effluent treatment (Chaukura *et al.*, 2017)

Due to its complicated nature, it is difficult to degrade synthetic dyes (Rubeena *et al.*, 2018) Half of the dye sprayed is not deposited on the fibers of the fabric and finally ends

up in wastewater. For every ton of textile products, 350-380 m^3 of water is utilized, which accounts for 15% to 20% of industrial pollution.(Kishor *et al.,* 2021)

Dye removal from water and wastewater can be handled by physical-chemical processes such as adsorption, advanced oxidation processes (AOPs), coagulation, membrane processes, and chemical precipitation. Reactive dyes in particular are among the organic pollutants that AOPs have shown to be effective at mineralizing from water and wastewater (Nidheesh *et al.*, 2015, Xavier *et al.*, 2015 and Ghalebizade *et al.*, 2019)

In wastewater treatment, ozone (O_3) is frequently employed as a disinfectant or oxidant. O_3 and hydroxyl radicals formed during O_3 breakdown can oxidize contaminants. According to several studies O_3 is particularly successful at removing complex dye molecules from a variety of water matrices(X An *et al.*, 2019).

The peroxide technique is a viable and environmentally beneficial solution (Khuntia *et al.*, 2011, Dubey *et al.*, 2021 and Mohan *et al.*, 2022) Commercially available activated carbon is frequently and effectively used as an adsorbent to remove dye from wastewater but it is expensive and has issues with regeneration (Gaya *et al.*, 2009 and Popuri *et al.*, 2015).

Therefore, using inexpensive, locally accessible materials to absorb dyes is a desirable alternative (Ramam *et al.,* 2016). Waste coir pith, pine sawdust, orange peel, agricultural by-products, Indian rosewood sawdust and banana pith are materials that have recently been used for this purpose (Kara *et al.*, 2007, Paraskeva *et al.*, 2008, Quan et al., 2020, Deshmukh *et al.*, 2018, Yiliusman *et al.*, 2018 and Ukanwa *et al.*, 2019). Studies have been carried out to investigate the effect of current intensity, electrolyte concentration and about the removal of the azo dye before releasing the dye into the wastewater (Lach *et al.,* 2022). The isolated bacterial strains in the consortium were used to decolorise the mixed azo dyes (Al-Ansari *et al.,* 2022).

Six microbial strains from soil contaminated with textile waste effluents and evaluated to reduce chemical oxygen demand (COD) and biological oxygen demand (BOD) levels of textile waste effluents (Bhaumik *et al.,* 2013). Bacterial species (Bacillus sp., Pseudomonas sp., and Alcaligenes sp.) and two fungal species (Aspergillus sp., and Penicillium sp.) to reduce chemical oxygen demand (COD) of textile effluents (Kim *et al.,* 1997).

In 2020, the performance of microalgae *Chlorella vulgaris* in an *Enterobacter* sp. MN17-assisted textile industry wastewater treatment system was evaluated for decolorization, removal of heavy metals (Cu, Cr, Pb, and Cd) and chemical oxygen demand (COD) (Chequer *et al.,* 2009).

The nanoparticles demonstrated the ability to degrade crystal violet with effective decrease in BOD and COD of the wastewater under optimized conditions. Taken together, the results of the present study show a prospective role of AgNPs using *Trigonella* seeds as an environmentally benign treatment approach for domestic/textile industry wastewater (Dutta *et al.,* 2022)

Various types of isotherms like Freunlich, Langmuir, and Temkin isotherms are studied for the adsorption of dyes. Among these Langmuir isotherm indicated that the capacity for the adsorption of cationic dyes was higher than that for anionic dyes (Zhang *et al.,* 2023).

Langmuir isotherm was observed and suggested that both homogeneous and heterogeneous sites are involved in accomplishing the process of adsorption of malachite green (Hahladakis *et al.,* 2013). Langmuir model and the Freundlich equation was compared and it was concluded that the Freundlich model fitted well for the dye Rhodamine b. and the Langmuir model fitted well dye Congo red (Machado *et al.,* 2013).

2.7 Nanoparticles

Due to their exceptional qualities and diverse applications, nanomaterials are frequently used. They are distinguished by their high surface area. Machado examined the capacity to produce nano zerovalent iron in leaf extract from 26 different plants.

The significance of synthesis variables such as the leaf mass to solvent volume ratio, extraction temperature, and extraction time was also examined (Wan *et al.,* 2022). The extract of *Chrysanthemum* flower was used as a botanical source for the production of Ag NPs and its potential use in bacterial reduction and azo-contaminated wastewater treatment is revealed by (Mahajan *et al.,* 2012).

The coated cotton fibres were further investigated for antibacterial, antiviral, and antifungal analysis. The study revealed that herb-encapsulated nanoparticles can be used in numerous applications in the field of medical textiles. Different metal

(Ag/Au/Zn/Cu/Pt/Pd/Ti) and metal oxide ($ZnO/CuO/SiO_2/TiO_2/Fe_2O_3$) nanoparticles make up inorganic nanoparticles.

Metallic nanoparticles (metal and metal oxide) have a small size, high loading capacity, and great stability when compared to organic nanoparticles (Mahajan *et al.*, 2012).

The most popular techniques for creating metallic nanoparticles (NP) are top-down and bottom-up methods, which aim to achieve nanostructures with specific functionalities by fine-tuning the physicochemical properties (Zhu *et al.*, 2014).

2.7.1 Top-down approach:

In this strategy, macro-crystalline bulk materials are broken down into nano-sized structures using mechanical force. Nanostructures are created using a variety of techniques such as milling, pyrolysis, etching, laser ablation, sputtering etc (Pandey *et al.*, 2016).

2.7.2 Bottom-up approach:

This strategy focuses on the self-assembly of atoms and molecules into clusters, which eventually results in the synthesis of desired homogeneous metallic nanoparticles (Iravani *et al.*, 2014). This method can be roughly divided into chemical and biological ways of producing nanoparticles.

Various nano adsorbents can be used to adsorb heavy metals. Some of them are listed below in Table 2.

Table 2 - Nanoadsorbents and their Adsorption Capacities

ADSORBENT	HEAVY METAL	MAXIMUM ADSORPTION CAPACITY (mg g^{-1})	REFERENCES
Graphene oxide	Pb^{2+}	35.6	Li *et al.*, 2015
Polysulphone/hydrous ferric oxide NPs	Pb^{2+}	13.2	Mohan *et al.*, 2018

Magneticbiochar	Pb^{2+}	0.11	Karunanayake *et al.*, 2018
Pinewood char	Pb^{2+}	4.13	Mohan *et al.*, 2018
CNTs	Pb^{2+}	17.44	Gonawala *et al.*, 2014
Activated carbon	Pb^{2+}	21.2	Stafiej *et al.*, 2007
L-Cysteine functionalised Fe_3O_4 NPs	Pb^{2+}	18.8	Shi *et al.*, 2019
Mesosporous magnetite (Fe_3O_4)	Pb^{2+}	19	Babgi *et al*, 2016

2.8 Green synthesis of nanoparticles

There have been reports of green nanoparticle manufacturing employing various plants as reducing, capping, and stabilizing agents. To prevent environmental damage, monitoring the effluent caused by exposure to textile wastewater should be done using a multidisciplinary approach that includes chemical and biological approaches.

Green synthesis of silver nanoparticles was done from the leaf extract of *Vitex negundo* and root extract of *Berberis aristata.*Silver nanoparticles from the seed extract of *Olea europaea* have been synthesized (Otari *et al.*, 2014).

Zanthoxylum has stomachic, carminative, and anthelmintic characteristics. It has been widely employed in many indigenous medicinal practices to treat a number of ailments. It has numerous uses in Ayurveda, allopathy, general pharmacy, and other fields. It has a very high trade value. It has insecticidal or larvicidal, antimicrobial, antiviral, antioxidant, anti-inflammatory, or cytotoxic effects.

In my work, nanoparticles using the seeds of *Zanthoxylum* will be prepared and will be used as a reducing and capping agent for the formation of metal nanoparticles. They can be then used as an efficient adsorbent for the removal of Methylene blue.

2.9 Literature summary

A review of the literature indicates that there are a number of strategies for the efficient use of wastewater by low-cost methods using different industrial wastes as an absorbent for the efficient treatment of textile wastewater. An extensive search was made for waste treatment methods that would be economical and environmentally benign.

The discharge of effluents from the textile industries of Ludhiana and their impact on human health is a major concern, therefore the goal of the current study is to examine the physiochemical properties of textile effluent that drains into Buddha Nullah of district Ludhiana, Punjab.

2.10 Research gap

The majority of the studies done on the Buddha Nullah focus on the prevailing issues, although the data indicates that the number of industries on the Buddha Nullah bank is still rising. The state government and its different agencies have made numerous attempts over the past 30 years to reduce pollution, but the pollution levels have only become worse.

To prevent environmental damage, it is important to monitor the changes in the effluent attributes caused by exposure to textile wastewater using a multidisciplinary approach that incorporates chemical and biological parameters. According to the literature survey, there are several methods for effectively treating textile wastewater using low-cost technologies. However, there hasn't been much effort done to create nanoparticles from seed extract of *Zanthoxylum armatum* that can break down dyes and lessen heavy metal pollution.

2.11 Problem identification

According to well-known environmentalist Sant Balbir Singh Seechewal, the Buddha Nullah is the most dangerous and toxic body of water in Punjab and it has an adverse effect on the lives of over 2 million people in both Punjab and Rajasthan. Sadly, it was once a stream with glistening water that served as "the center of social life in Ludhiana."

Raw industrial effluents from 228 dyeing operations, raw sewage waste from Ludhiana's STPs and 16 "outlets" that directly discharge sewage and industrial waste into the creek account for the majority of the pollution in Buddha Nullah. Buddha nullah is responsible for 90% of the Sutlej River's pollution.

According to a Punjab Agricultural University study, crops and vegetables grown in localities along the nullah include carcinogens including copper, chromium, cadmium, mercury, and others. Near the Sutlej confluence, the river's water turns Class E unclean, making it unfit for any use and incapable of supporting any aquatic life.

More than two million people in Punjab and Rajasthan are impacted by Buddha Nullah, the largest reservoir of contaminated water in Punjab. Sadly, it used to be a spotless stream and the hub of social activities in Ludhiana.

In 2010, water testing from Buddha Nullah revealed quite high levels of heavy metals as well as uranium concentrations that were 1.5 times the acceptable range. Chromium, for instance, was 50 times more than the reference range. Silver, manganese and lead concentrations were all high, while aluminium and iron concentrations were 20 and 60 times higher.

The Ludhiana district administration began in situ bioremediation in April 2011 to reduce contamination in Buddha nullah, but the stream's water was so poisonous that even the bacteria introduced during the biological cleaning procedure did not survive.

Since India already has the greatest water contamination, any further delay in adopting mitigation measures will only have a negative impact on food production, biodiversity and the health of the reliant population. The 840-crore revitalization project that was scheduled to transform Buddha Nullah into Buddha River missed four deadlines to finish the capital construction due to teething issues.

According to April 2019 reports of water testing, upstream of the confluence with Buddha Nullah, the river's BOD concentration was found to be in the range of 1.0 to 3.0 mg/l, but 100 meters downstream of the confluence, Sutlej's BOD surges abruptly to 90 mg/l. The river's water reaches Class E status in terms of pollution at the Sutlej confluence, making it unfit for any use and devoid of all aquatic life.

While working on a project to rejuvenate Buddha Dariya on July 1, 2023, the municipal corporation (MC) discovered illegal sewage pipelines installed to transfer untreated dye industry effluent from the Common Effluent Treatment Plant (CETP) into MC sewer systems on Tajpur road. The evidence showed that PDA had built the lines to forcibly dump waste from the dyeing industry into the MC sewer lines. No significant investigation into the water quality in Buddha Nullah has been conducted since 2010. Despite spending tens of millions of dollars, the pollution crisis has not subsided. Hence, there is a need to look into the issue and offer a remedy because the quality of the water is still declining.

Based on a review of the literature, it can be said that the physico-chemical examination of textile effluents from the industrial region of Ludhiana and their subsequent discharge into the Buddha Nallah are serious issues. My research will therefore be primarily focused on determining the level of contamination brought on by textile effluents in the Ludhiana industrial region.

The present investigation will be carried out in three phases:

PHASE I

Green synthesis of silver nanoparticles using seed extract of *Zanthoxylum armatum* will be done. Characterisation of nanoparticles will be done using techniques like X-ray diffraction (XRD), Ultra-voilet Spectroscopy (UV), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Also the kinetic study of silver nanoparticles using adsorption isotherms will be carried out.

PHASE II

Nanoparticles will be used to degrade the dye and remove the heavy metals from the textile effluents.

PHASE III

Nanoparticles prepared from the microbial strains as well as *Zanthoxylum armatum* will be used for the treatment of dyes and removal of heavy metals.

2.9 Problem statement

Synthesis and characterisation of silver nanoparticles using seed extract of *Zanthoxylum armatum* for remediation of dye & heavy metal contaminated textile effluents.

2.10 Objectives

1. Green synthesis and characterization of silver nanoparticles using seed extract of *Zanthoxylum armatum* and their kinetic studies.

2. Application of plant mediated silver nanoparticles for the degradation of dyes in the textile effluents.

3. Removal of heavy metals in the effluents from textile industries using plant mediated silver nanoparticles.

4. To study the combined effect of silver nanoparticles and microbial strains for the treatment of dyes and removal of heavy metals.

3. MATERIALS AND METHODS

3.1 Sampling area

Zanthoxylum armatum varies in habit from a prickly, deciduous to an evergreen shrub, woody climber, or small tree growing up to 5 meters. A plant has a wide environmental tolerance as it is found in temperate or tropical zones. It grows well in deep moisture-retentive soil. Flowers are formed on the old wood. They are Dioecious. Male and female plants must be grown if seed is required. The plant mainly grows in the wild. It is sometimes also cultivated and is grown as a hedge in northern India. The seeds of *Zanthoxylum armatum* will be collected from the forest area of Solan.

3.2 Objective of sampling

The current research will examine the presence of harmful dyes and heavy metals in the textile manufacturing waste from Ludhiana, Punjab as well as from the adjoining Buddha Nallah River, which has an impact on the development of aquatic life and flora where more than two million residents of Punjab and Rajasthan are impacted. It's a shame because the stream once used to be immaculate and the hub of social activities in the city of Ludhiana.

Scanning electron microscopes can image individual nanoparticles to characterize their shape, size, and location. Advances in scanning electron microscopy (SEM) enable the high-resolution imaging of single nanoparticles (NPs) with sizes well below 10 nm. The SEM analysis is well suited for in-depth and, particularly valuable, to very accurate, traceable, lateral dimensional measurements of NPs.

3.3 Synthesis of silver nanoparticles

Zanthoxylum armatum is a fragrant, deciduous, prickly shrub can reach a height of 3.5 meter. *Zanthoxylum armatum* is also referred to as prickly ash, is a traditional remedy for fever, discomfort, and inflammation. Since ancient times, it has been used as medicine to treat a variety of illnesses, including gum bleeding, fever, dyspepsia, toothaches, and dental-related issues. It is also used as a tonic.

The seeds of *Zanthoxylum armatum* contain alkaloids, flavonoid, tannins, phenolic terpenoids and their glucosides and benzenoids, fatty acids and amino acids which are responsible for the synthesis and capping of nanoparticles. Due to their special qualities,

silver NPs can be used in antibacterial applications, biosensor materials, composite fibers, cryogenic super-conducting materials, cosmetic items and electrical components, among other uses.Silver nanoparticles will be prepared using the seed extract of the *Zanthoxylum armatum*. 1 mM aqueous solution of silver nitrate ($AgNO_3$) will be prepared and used for the synthesis of AgNPs.

Synthesis of AgNPs from silver nitrate solution takes place by co-precipitation using seed extract of *Zanthoxylum armatum* which acts as reducing and capping agent (M Devi *et al.*, 2020).The nanoparticles will be characterised by UV-visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques.

3.4 Synthesis of nanoparticles from microbial strain

A suitable microbial strain will be procured from IMTECH, Chandigarh and silver nanoparticles will be prepared. Bacteria can be the right choice because they grow faster than other biological systems such as plants or fungi, and the growth conditions can be controlled. Moreover, bacterial cultures are relatively inexpensive. AgNPs will be synthesized using suitable microbial strains. AgNPs will be used for heavy metal and dye removal applications. Characterisation of nanoparticles will be done using techniques using UV-spectroscopy, FE-SEM and TEM. The characteristics determined using these techniques are listed below in Table 3.

Table 3 - Characterisation techniques

TECHNIQUES	CHARACTERISTIC
X RAY DIFFRACTION(XRD)	Average particle size of nanoparticles
SCANNING ELECTRON MICROSCOPY(SEM)	Aggregation, size and shape
TRANSMISSION ELECTRON MICROSCOPY(TEM)	Aggregation, size and shape
UV-VIS/Infrared spectroscopy	Structure, Functional group analysis

BET Analysis	Surface adsorption studies

CONCLUSION

The textile industry is one of the largest consumers of potable water and consequently, produces a huge amount of wastewater. The typical characteristics of textile factory wastewater have already been documented previously.

The real effluents from different textile mills presented in studies vary widely and some of the characteristic parameter values were more than the permissible limits. Since the presence of dyes and heavy metals have deleterious effects on our health so they must be removed prior to disposal.

Immobilizing bacterial cells coupled with AgNPs is an association between nanotechnology and biotechnology. It is employed to combine the benefits of biological treatments by utilizing bacterial strains with the capacity to decolorize and break down azo dyes. The silver nanoparticles are reusable without losing their efficiency. It is a quick and accurate process. So remediation will be done using silver nanoparticles prepared from the seeds of *Zanthoxyllum armatum* and suitable bacterial strains.

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SUGGESTIONS BY RESEARCH DEGREES COMMITTEE (RDC)

1. Preparation of silver nanoparticles has been included as the main objective.
2. The number of objectives has been reduced from 5 to 4.
3. Characterisation of dye effluents was removed from the list of objectives. This included analysis of physiochemical parameters.
4. Kinetic study of silver nanoparticles using adsorption isotherms was included in the objectives.
5. BET analysis was included in the list of characterisation techniques.

To,

The Chairperson

Department of biotechnology

Himachal Pradesh University

Shimla -171005 H.P. (India)

Subject: To seek permission to use lab facilities for the preparation of silver nanoparticles.

My name is Anju Bhardwaj and I am pursuing PhD (Chemistry) at Baddi University. I need to prepare Ag nanoparticles using seed extract of *Zanthoxylum armatum* to study the combined effect of Silver nanoparticles and Microbial strains for the treatment of dyes and removal of heavy metals from textile effluents.

You are requested to permit me to work in your RL-V lab and utilise the lab facilities for research work.

Thank you for considering our request. I look forward to hearing from you soon.

Regards

Anju Bhardwaj

(BUPIN 22DCH001)

On Friday, September 8, 2023 at 09:24:38 PM GMT+5:30, hod ash <hod.ash@baddiuniv.ac.in> wrote:

Sir

As per our telephonic conversation, you are requested to allow Ms.Anju Bhardwaj, pursuing Ph.D at Baddi University, to use the research facilities of your laboratory for the preparation of nanoparticles.

Her request letter to seek permission is attached herewith.

Thanks and regards
Dr.Nidhi Gupta

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Regards
Dr. Nidhi Gupta
Associate Dean - School of Sciences
Baddi University of Emerging Sciences and Technology
Makhnumajra, Baddi
Mobile: +919896580077

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Regards
Dr. Nidhi Gupta
Associate Dean - School of Sciences
Baddi University of Emerging Sciences and Technology
Makhnumajra, Baddi
Mobile: +919896580077

On Monday, September 11, 2023 at 11:57:44 AM GMT+5:30, hod ash <hod.ash@baddiuniv.ac.in> wrote:

Greetings of the day Sir!!

As desired, I am sending the request letter on the official letterhead. Kindly check.

On Sat, Sep 9, 2023 at 10:52 AM hod ash <hod.ash@baddiuniv.ac.in> wrote:
Good morning sir!!

Sure Sir.I will mail you the request letter on the official letterhead on Monday positively.

Thankyou so much sir for your response and support.

Regards

On Fri, Sep 8, 2023, 11:30 PM Arvind Bhatt <bhtarvind@yahoo.com> wrote:
Thank you so much. May I request you to send letter 9n your official letterhead?

Yours Sincerely,

Professor (Dr.) Arvind Kumar Bhatt
Department of Biotechnology
Himachal Pradesh University,
Shimla-171 005-HP (India)
Dean- Planning and Teachers Matters
Incharge - Biotechnology Incubator, HP University, Shimla
Secretary - Association of Biotechnologists of Himachal Pradesh (ABHI)
Treasurer - Indian Science Congress Association (ISCA), Shimla Chapter
President - Vigyan Bharti, Himachal Pradesh,
Associate Editor - HP University Research Journal
Convener - Environment Cell - IQAC
Member - State Level Task Force on Biotechnology, HP
Ex-Director- Institute of Integrated Himalayan Studies (IIHS), HP University, Shimla
Ex-Coordinator, UIIT, HP University, Shimla
Ph: 91-177-2831948, 91-177-2833705, (D), +94184-50009
E-Mail: bhtarvind@yahoo.com, arvind.bhatt@hpuniv.ac.in, bhattak08@gmail.com,
Website: www.hipuniv.ac.in/biotech

---------- Forwarded message ---------
From: **hod ash** <hod.ash@baddiuniv.ac.in>
Date: Wed, 21 Feb 2024 at 2:53 PM
Subject: Fwd: Permission to use your lab facilities for research work
To: <anjubhardwaj1385@gmail.com>

---------- Forwarded message ---------
From: **Arvind Bhatt** <bhtarvind@yahoo.com>
Date: Wed, Feb 21, 2024 at 2:47 PM
Subject: Re: Permission to use your lab facilities for research work
To: hod ash <hod.ash@baddiuniv.ac.in>

Dear Dr. Nidhi Gupta,

In response to your email I wish to inform you that Ms. Anju Bharadwaj may use the laboratory facilities for her research work after consulting the researchers as per their convenience. However, she may have to provide the chemicals required exclusively for her work.

Further, since the University already has the scarcity of hostels, she will have to arrange accommodation herself.

Further she may fix with the researchers in my lab for starting her research work.

With regards

Yours Sincerely,

Professor (Dr.) Arvind Kumar Bhatt
Department of Biotechnology,
Himachal Pradesh University, Shimla-171 005-HP (India)
Nodal Officer I/C - Biotechnology Incubation Centre, HPU Shimla
President - Association of Biotechnologists of Himachal Pradesh (ABHI)
Member - State Level Task Force on Biotechnology, HP
Formerly:
Dean - Planning and Teachers Matters
Head - Departments of Biotechnology, Microbiology & Forensic Science, HP University
Treasurer - Indian Science Congress Association (ISCA), Shimla Chapter
President - Vigyan Bharti, Himachal Pradesh
Director- Institute of Integrated Himalayan Studies (IIHS), HP University, Shimla
Coordinator, UIIT, HP University, Shimla
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